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# REACTION BETWEEN 1-ARENESULPHONYL-3-NITRO-1,2,4-TRIAZOLES AND NUCLEOSIDE BASE RESIDUES. ELUCIDATION OF THE NATURE OF SIDE-REACTIONS DURING OLIGONUCLEOTIDE SYNTHESIS

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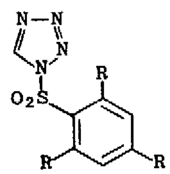
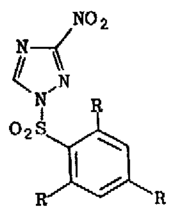
Summary. The protected guanosine and uridine derivatives (*3a*, *3b* and *4*) react with 1-(mesitylene-2-sulphonyl)-3-nitro-1,2,4-triazole (MSNT, *2a*) to give *5a*, *5b* and *7*, respectively, *9* is converted into *10* in the same way. The reactions proceed more rapidly in the presence of diphenyl phosphate.

The key step in the phosphotriester approach[1] to oligonucleotide synthesis consists of the activation of a phosphodiester group and the subsequent phosphorylation of a hydroxy function to form a new internucleotide linkage. The most suitable condensing agents with regard to short reaction times and high yields of products appear to be arenesulphonyl derivatives of tetrazole (putative structures, *1*), introduced by Narang and his coworkers[2] and arenesulphonyl derivatives of 3-nitro-1,2,4-triazole (putative structures, *2*) later introduced by us[3].



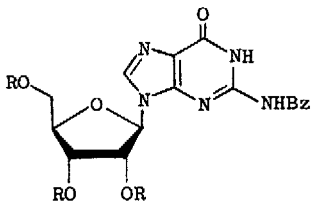
*1*



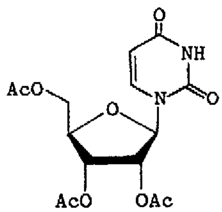
*2* *a*, R = Me
*b*, R = $Me_2CH$

Recently, in studies directed towards the synthesis of yeast alanine transfer ribonucleic acids (tRNA), we observed[4] side-reactions leading to mixtures of products and diminished yields when a large excess of 1-(mesitylene-2-sulphonyl)-3-nitro-1,2,4-triazole (MSNT, *2a*) was used as the condensing agent and when phosphorylation reactions were allowed to proceed for a relatively long time. With the intention of elucidating the nature of the side-reactions, we have examined the reactions between MSNT (*2a*) and appropriate nucleoside derivatives. We have also examined the effect of an added phosphodiester component on the course of the side-reactions

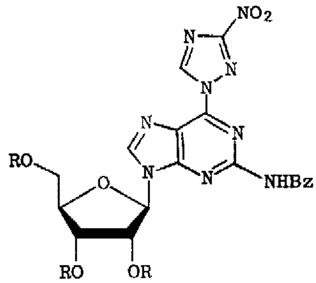
We first investigated the action of MSNT (*2a*) on *N*-acyl-2',3',5'-tri-*O*-acyl derivatives of adenosine, cytidine and guanosine and on 2',3',5'-tri-*O*-acetyluridine (*4*) While there was no detectable reaction between MSNT (*2a*, 5 molecular equivalents) and 2-*N*-benzoyl-2',3',5'-tri-*O*-acetyladenosine or 2-*N*-(*p*-anisoyl)-2',3',5'-tri-*O*-(*p*-anisoyl)cytidine in pyridine solution after 24 hr even in the presence of diphenyl phosphate (0.5 molecular equivalents, see below), MSNT (*2a*) was found to react readily with 2-*N*-benzoyl-2',3',5'-tri-*O*-acetylguanosine (*3a*) and 2',3',5'-tri-*O*-acetyluridine (*4*), especially in the presence of diphenyl phosphate.



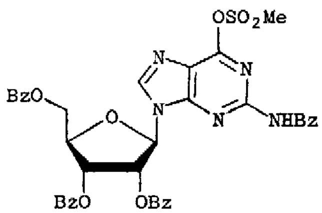
*3* *a*, R = Ac
*b*, R = Bz



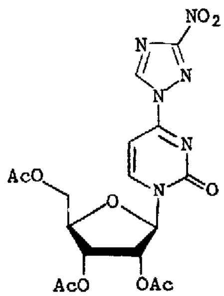
*4*



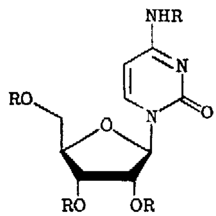
*5* *a*, R = Ac
*b*, R = Bz



*6*



*7*



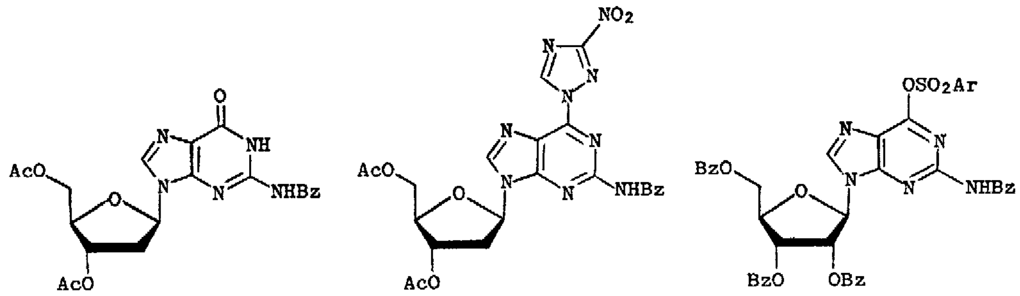
*8* *a*, R = H
*b*, R = Bz

The time for half-completion ($t_{1/2}$) of the reaction between 2-*N*-benzoyl-2',3',5'-tri-*O*-acetylguanosine (*3a*, 0.1 mmol) and MSNT (*2a*, 0 51 mmol) in pyridine (0.5 ml) solution at room temperature was found to be *ca*. 48 hr but the reaction proceeded more rapidly ($t_{1/2}$ ∿ 25 and 9 hr) when diphenyl phosphate (0 01 and 0 05 mmol, respectively) was added to the reaction mixture. Following a preparative scale reaction, a crystalline compound, m.p. 193°C, was isolated from the products in 71.5% yield and identified as *5a* on the basis of analytical and spectroscopic evidence[5] Confirmation of this structural assignment was provided by an independent synthesis of the corresponding tetrabenzoyl derivative (*5b*) which was isolated as a crystalline solid, m p 138°C, in 82% yield from the products of the reaction between the 6-*O*-mesyl derivative[6] (*6*) and 3-nitro-1,2,4-triazole in pyridine-dioxan solution at room temperature. The identical tetrabenzoyl derivative (*5b*) was obtained in 69% yield by treating 2-*N*-benzoyl-2',3',5'-tri-*O*-benzoylguanosine (*3b*) with an excess of MSNT (*2a*) in the presence of diphenyl phosphate.

The time for half-completion of the reaction between 2',3',5'-tri-*O*-acetyluridine (*4*, 0.11 mmol) and MSNT (*2a*, 0.51 mmol) in pyridine (0.5 ml) at room temperature to give *7* was found to be 25 hr and again the reaction proceeded more rapidly ($t_{1/2}$ ∿ 7 and 3 hr) when diphenyl phosphate (0 01 and 0.05 mmol, respectively) was added to the reaction mixture. Following a preparative scale reaction, *7* was isolated as a crystalline solid, m p. 89°C, in

78.5% yield and characterized on the basis of analytical and spectroscopic evidence[7] When *7* was treated with aqueous ammonia (*d* 0 88)-dioxan (2:1 v/v) for 16 hr at room temperature, cytidine (*8a*) was obtained as the sole nucleoside product and was then isolated as its crystalline tetrabenzoyl derivative (*8b*) in 63 5% yield.

With the intention of identifying possible side-reactions in oligodeoxyribonucleotide synthesis, we next examined the action of MSNT (*2a*) on 2-*N*-benzoyl-3',5'-di-*O*-acetyl-2'-deoxyguanosine (*9*) and 3',5'-di-*O*-acetylthymidine. The *N*-benzoylguanine residue in *9* was modified in the same way (and at virtually identical rates under the same conditions both in the absence and presence of diphenyl phosphate) as it was in *3a* and, in a preparative scale experiment, *10* was obtained as a crystalline compound, m.p. 108-110°C, in 70% isolated yield. However, the reaction between MSNT (*2a*) and 3',5'-di-*O*-acetylthymidine was extremely slow and only a trace of product was obtained under the conditions required for the virtually total conversion of 2',3',5'-tri-*O*-acetyluridine (*4*) into *7*




*9* *10* *11* Ar = 2,4,6-$Me_3C_6H_2$

The mechanism of the formation of the nitrotriazole derivatives (*5*, *7* and *10*) has not yet been elucidated but if, for example, it is assumed that *3b* reacts first with MSNT (*2a*) to give 3-nitro-1,2,4-triazole and the 6-*O*-(mesitylene-2-sulphonyl) derivative (*11*), corresponding to the 6-*O*-mesyl derivative (*6*), then the conjugate base of 3-nitro-1,2,4-triazole would be expected to react with *11* to give *5b*. It has been found[3] that the combination of MSNT (*2a*) and a phosphodiester (e.g. diphenyl phosphate) constitutes a very powerful phosphorylating system for alcoholic hydroxy functions. However, although diphenyl phosphate appears to catalyze the formation of the nitrotriazole derivatives (*5*, *7* and *10*), it does not follow that the 2-*N*-benzoylguanine and uracil residues are initially phosphorylated rather than sulphonated (to give, for example, *11*) by the combination of MSNT (*2a*) and diphenyl phosphate. No intermediates could be detected (by t.l.c.) in the conversions of *3a*, *3b*, *4* and *9* into *5a*, *5b*, *7* and *10*, respectively.

The results of this investigation will be of considerable help in determining the most effective strategy for oligonucleotide synthesis when MSNT (*2a*) is used as the condensing agent. If the side-reactions are to be minimized, it is important that (i) the use of an excess of the phosphodiester component should be avoided, (ii) the use of a large excess of MSNT (*2a*) should be avoided, and (iii) condensation reactions should be as brief as possible. While considerable care should be taken in the synthesis of oligoribonucleotides as uracil residues (see above) are particularly susceptible to modification, it is also advisable to take the above three points into account when the synthesis of oligodeoxyribonucleotides is being undertaken. However, it is likely that the modification of uracil and 2-*N*-benzoylguanine residues will be reversed during the unblocking of protected oligonucleotides with *syn*-4-nitrobenzaldoximate ion[3b]. Thus, treatment of *5b* and *7* with a slight excess of 0.5 *M*-$N^1,N^1,N^3,N^3$-tetramethylguanidinium *syn*-4-nitrobenzaldoximate[3b] in dioxan solution at room temperature for 30 min led to the quantitative regeneration of the corresponding guanosine and uridine derivatives (*3b* and *4*, respectively). The latter compounds were isolated from the products as crystalline solids in 77 and 74% yields, respectively. The ready transformation (see above) of *7* into cytidine (*8a*) by ammonolysis[8] reveals one of the pitfalls of using ammonia[9] to remove aryl protecting groups from internucleotide linkages in oligonucleotide synthesis.

The occurrence of side-reactions is not limited to condensation reactions involving MSNT (*2a*)[10]. Thus, 1-(2,4,6-tri-isopropylbenzenesulphonyl)-3-nitro-1,2,4-triazole[3a] (TPSNT, *2b*) also reacts with *3a* and *4* to give *5a* and *7*, respectively, but the reactions proceed at approximately one-half of the rates of the corresponding reactions with MSNT (*2a*). As condensation reactions involving TPSNT (*2b*) also proceed more slowly, it is anticipated that the extent of base modification would be at least as great if the latter reagent (*2b*) were used instead of MSNT (*2a*) in oligonucleotide synthesis. Finally, preliminary experiments suggest that the mesitylene-2-sulphonyl derivative of tetrazole (*1*, R = Me)[2] reacts at a greater rate than MSNT (*2a*) with uracil and 2-*N*-benzoylguanine residues and that the reactions are more complex

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## REFERENCES AND FOOTNOTES

[1]C.B. Reese, Tetrahedron Report No. 56, *Tetrahedron* 34, 3143 (1978).

[2]J. Stawinski, T. Hozumi, and S.A. Narang, *Can. J Chem.* 54, 670 (1976).

[3](a) Y.T. Yan Kui, Ph.D. Thesis, London University, 1977, pp 146-155, (b) C.B. Reese, R.C. Titmas, and L Yau, *Tetrahedron Letters* 2727 (1978)

[4]S.S. Jones, B. Rayner, C.B. Reese, A Ubasawa, and M. Ubasawa, *Tetrahedron* 36, in the press (1980).


[5]Satisfactory microanalytical data were obtained for all new compounds described. The spectroscopic data obtained for *5a* [$^1$H n.m.r (90 MHz, $d_6$-DMSO) $\delta$ 2.00 (3H,s), 2.08 (3H,s), 2.13 (3H,s), 4 45 (3H,m), 5.75-6.1 (2H,m), 6 39 (1H, d, $J$ = 4 4 Hz), 7.35-7.75 (3H,m), 7.9-8.1 (2H,m), 8 92 (1H,s), 9.85 (1H,s), 11.48 (1H, br.s); u.v. (95% EtOH) $\lambda_{max}$ 315, 249 ($\varepsilon$ 9,800, 29,000), $\lambda_{min}$ 302, 223 nm ($\varepsilon$ 9,100, 21,000)] did not allow an alternative formulation as an isomeric 2-nitro-1,3,4-triazole derivative to be excluded, *5a* had $R_F$ 0.34 [$CHCl_3$-MeOH (19:1 v/v)].

[6]P.K. Bridson, W. Markiewicz, and C B. Reese, *J C.S. Chem. Comm.* 447 (1977).

[7]The n.m.r [$^1$H (90 MHz, $d_6$-DMSO) $\delta$ 2.08 (6H,s), 2.11 (3H,s), 4.36 (3H,m), 5.38 (1H, t, $J$ = 6 Hz), 5 59 (1H, dd, $J$ = 3 and 6 Hz), 6.04 (1H, d, $J$ = 3 Hz), 7.15 (1H, d, $J$ = 7 Hz), 8.59 (1H, d, $J$ = 7 Hz), 9.75 (1H,s)] and u.v. [(95% EtOH) $\lambda_{max}$ 323, 249 ($\varepsilon$ 6,400, 16,000), $\lambda_{min}$ 290, 228 nm ($\varepsilon$ 2,900, 12,000)] spectroscopic data obtained for *7* again did not allow an alternative formulation as an isomeric 2-nitro-1,3,4-triazole derivative to be excluded, *7* had $R_F$ 0.29 [$CHCl_3$-MeOH (19 1 v/v)].

[8]This result also suggests that *7*, which may readily be prepared in two steps from uridine, might prove to be a valuable intermediate in the synthesis of, for example, cytidine derivatives.

[9]J. Stawinski, T. Hozumi, S A Narang, C.P. Bahl, and R. Wu, *Nucleic Acids Res.* 4, 353 (1977).

[10]In their work on the synthesis of oligodeoxyribonucleotides by the phosphotriester approach, Gough *et al.* [G.R. Gough, K.J. Collier, H.L. Weith, and P.T. Gilham, *Nucleic Acids Res.* 7, 1955 (1979)] have recently observed significant quantities of by-products in reactions involving 2-*N*-isobutyrylguanine residues when 1-tosyl-4-nitroimidazole (TSNI) is used as the condensing agent. These workers used 1.3 molecular equivalents of the phosphodiester component and 4.0 molecular equivalents of TSNI with respect to the component with the 5'-hydroxy function, and allowed the reactions to proceed for 16-18 hr. However, as the side-reactions observed were reversed by a short treatment with aqueous pyridine, the products would seem not to correspond to 4-nitroimidazole equivalents of *10* (with 2-*N*-benzoyl replaced by 2-*N*-isobutyryl residues)

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